that provide subdiffraction optical resolution [3]to study the relation of biomolecular structure and function of TNFa binding to the receptor at the nanometer scale.

References:
[1] **Baltimore D.** Multiple nuclear factors interact with the immunoglobulin enhancer sequences. Cell. 1986 Aug 29;46(5):705-16.
[2] **Widera D, Kaltschmidt C, Kaltschmidt B.** Tumor necrosis factor alpha triggers proliferation of adult neural stem cells via IKK/NF-kappaB signaling. BMC Neurosci. 2006 Sep 20;7:64
[3] **Heilemann M, van de Linde S, Schüttpelz M, Kasper R, Seefeldt B, Mukherjee A, Tinnefeld P & Sauer M.** Subdiffraction-Resolution Fluorescence Imaging with Conventional Fluorescent Probes. *Angew. Chemie,* **47**, 6172-6176. 2008

2566-Pos
Computational Modelling of the Drosophila Phototransduction Cascade
Konstantin Nikolic, Joaquim Loizou, Patrick Degenaar.
Imperial College London, London, United Kingdom.
This work presents detailed modelling of the single photon response, the quantum bump, of fly photoreceptors. All known components participating in the primary phototransduction process are taken into account, and estimates have been obtained for the both the physical and the chemical parameters. The result is a detailed analysis of the first, crucial step in fly vision. The same model can be used for multiphoton response, i.e. in the case of higher light intensity stimuli.
The model successfully reproduces the experimental results for the statistical features of quantum bumps (average shape, peak current average value and variance, the latency distribution, etc), arrestin mutant behaviour, low extracellular Ca cases, etc. The TRP channel activity is modelled using the Monod-Wyman-Changeux (MWC) theory for allosteric interaction, which led us to a physical explanation of how Ca/calmodulin regulates channel activity. The model can combine deterministic and stochastic approaches and allows for a detailed noise analysis. The computational model was coded in Matlab using the Parallel Computing Toolbox, which allows computations on multicore computers and computer clusters. An appropriate graphic user interface was developed which gives very convenient and instructive presentation of the parameters used in the modelling and could easily be expanded to other G-protein coupled cascade processes.

2567-Pos
Mathematical Model of Basal and Agonist-Dependent GIRK Channel Activity
Daniel Yakubovich, Shai Berlin, Nathan Dascal.
Tel-Aviv University, Sackler School of medicine, Tel-Aviv,
Ramat Aviv, Israel.
We developed a model of GIRK channel activity. The channel activation scheme was based on sequential non-cooperative binding of 4 Gβγ molecules to channel protein (generating 5 closed states) and Gβγ independent channel opening. The kinetics of Gβγ interaction with subsequent change of channel conformation were adjusted to generate activation time of ~ 1 s for a step rise in Gβγ concentration. The kinetics of switch from closed to open conformation were derived from single-channel analysis of GIRK1/2 recordings in *Xenopus leaves* oocytes. For simulation of agonist-dependent channel activation we incorporated the above scheme into a general model of G-protein cycle. This model was derived from that of Thomsen-Jaquez-Neubig. Several features were added: a) receptor was allowed to couple to G-protein in agonist-bound and in free state; b) finite affinity of Gα to Gβγ was assumed in GTP- and GDP-bound states; c) microscopic reversibility was obeyed in cyclic schemes containing reversible reactions; d) the assumption that G-protein concentration exceeded the receptor concentration was relaxed in order to enable simulation of titration experiments. We simulated the time-course of channel activation induced by step change in agonist concentration in presence and in absence of Gβγ-scavenging protein. We also simulated receptor-titration experiments. The results of simulations were compared to whole-cell experiments in *Xenopus leaves* oocytes. Our model produced realistic time course of channel activation and also demonstrated decremental dependence of activation time on receptor concentration. Comparing the simulation results with those expected from binary shuttle model of channel activation based on considerations of free diffusion of membrane proteins lead to the conclusion that G-protein activation by receptor is probably of catalytic collision-coupling type, while the channel and G protein were either in a tight complex or diffused in a restricted membrane domain.

2568-Pos
Electrophysiology and Live Fluorescence Imaging to Monitor the Effects of Potassium Channel Blockade on Lipopolysaccharide-Induced Immune Signaling
Adrian R. Boese Schiess[1], Elizabeth L. Carles[1], Jaclyn K. Murton[1], Bryan Carson[1], Catherine Branda[2], Conrad D. James[1], Susan B. Rempe[1].
[1]Sandia National Laboratories, Albuquerque, NM, USA, [2]Sandia National Laboratories, Livermore, CA, USA.
Studies have shown that ion-channel function in immune cells such as macrophages can influence pathogen-induced immune signaling. Thus, ion channels are viewed by some researchers as potential therapeutic targets for developing novel strategies for regulating immune response on demand when standard anti-pathogen therapies such as antibiotics and vaccinations fall short. However, the direct contribution of ion-channel function to the complex and interconnected signaling pathways in immune response has proved elusive, largely due to the difficulty in tracking multiple signaling nodes in these pathways in real-time. Toward this end, we tracked the real-time inflammatory response to *E. coli* derived lipopolysaccharide (LPS) in a mouse macrophage-like cell-line (RAW 264.7) with electrophysiology to measure potassium channel currents and live imaging with fluorescent fusion reporters of crucial events involved in immune signaling. We developed two reporter constructs: 1) GFP fused to the NFκB transcription factor subunit RelA (GFP-RelA) to track early (<30 min) immune response, and 2) a TNFα promoter driving expression of mCherry with a terminal PEST sequence construct to track later (>2 hours) cytokine induction. In RAW264.7 cells, a 100 nM LPS challenge produces two waves of GFP-RelA translocation from the cytoplasm to the nucleus while gradually increasing the expression of mCherry (TNFα promoter activity). Continuous exposure of LPS-challenged cells to the BK- and Kv-channel blocker tetraethylammonium modifies the translocation dynamics of GFP-RelA and the induction of the TNFα promoter in a dose-dependent manner. Thus we provide evidence in support of a BK- and/or Kv-channel contribution to both early and later LPS induced inflammatory signaling.

2569-Pos
Release of ATP Through Hemichannels Affects Basal Ciliary Activity in the Human Airways
Karla Droguett, Manuel Villalón.
Pontificia Universidad Católica de Chile, Santiago, Chile.
The frequency of ciliary beat (CBF) is the main factor that determines the effectiveness of mucociliary clearance in the airways. ATP is a known agonist of the CBF, since addition of ATP (10 μM) to the extracellular medium, increases the CBF in different ciliated epithelial cells. There is evidence that epithelial cells constitutively secrete ATP in the airways; however the contribution of extracellular ATP to the control of basal CBF has not been studied. We propose that the airway epithelium release ATP through hemichannels followed by an activation of purinergic receptors, contributes to the control of basal CBF. **Methods**: CBF was recorded using microphotodensitometry technique using primary cultures of human adenoid explants. We also used Western Blot analysis to determinate the expression of $P2Y_2$ purinergic receptor, Pannexin 1 and Connexin 43 hemichannels and used different channel blockers to determinate the contribution of each channel to the control of CBF. **Results**: The spontaneous basal CBF in the cultures was 9.3 ± 0.1 Hz (n=91) and the extracellular ATP concentration was 1.04 ± 0.36 nM in 1.5 mL (n=3). Apyrase (50 U/mL), an extracellular ATP ectonucleotidase, decrease the basal CBF in 19.4% ± 7.0 (n=7). Suramine, a purinergic receptor antagonist, reduce the basal CBF in a 12% and the hemichannels blockers 18β-Glycyrrhetinic acid (50 μM), Carbenoxolone (50 μM) and La^{3+} (100 μM), reduce the basal CBF in a 33.5% ± 4.9, 7.9% ± 1.3 and 21.74% ± 4.3 respectively (n=3). These results provide evidence that affecting the channels or hemichannels associated to the release of ATP or the paracrine/autocrine effects of ATP on the epithelium affects the CBF and suggest that extracellular ATP concentration might contribute to the control of basal CBF in the airways. FONDECYT 1080679.

2570-Pos
Metabotropic Purinergic Receptors in Satellite Glial Cells
Daniel R. Batista, Antonio C. Cassola.
Biomedical Sciences Institute - University of São Paulo, São Paulo, Brazil.
Objective: The dorsal root ganglia (DRG) contains the pseudo-unipolar neurons of sensory input. Neuron somata is enveloped by satellite glial cells (SGC) whose functions is still unknow. To further unveil the sinalization between neurons and glia in DRG we have investigated the expression of purinergic metabotropic receptors (P2Y) by SGC of DRG.